DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

0400 5 SEP 30 P2:14

Date: SEP 12 2005

From: Consumer Safety Officer, Division of Dietary Supplement Programs , Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification: PinnoThin™ FFA

Firm: Loders Croklaan B.V.

Date Received by FDA: June 22, 2005

90-Day Date: September 20, 2005

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Victoria Lutwak

19955-0316

RPT292

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

SEP 1 2005

Louis H.T. Dederen, M.Sc.
Safety and Regulatory Affairs Manager
Loders Croklaan B.V.
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

Dear Mr. Dederen:

This is to inform you that the notification, dated June 17, 2005 that you submitted pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was filed by the Food and Drug Administration (FDA) on June 22, 2005. Your notification concerned the substance that you identified as "PinnoThin™ FFA" that you intend to market as a new dietary ingredient for use in dietary supplement products. Additional information dated June 27 was received by FDA on June 27, 2005 identified the source of the ingredient as *Pinus koraiensis* Sieb. & Zucc.

According to your notification, "PinnoThin™ FFA is suitable as an ingredient in dietary supplements in various forms, including softgels, capsules and supplement bars and similar products." Your notification states that the recommended conditions of use will be to consume the ingredient to "provide a daily intake of 3.0 g PinnoThin™ FFA 30-60 min before the most substantial daily meal or 3.0 g of PinnoThin™ FFA between meals (i.e. not after the last meal)" and that "[t]he ingredient is intended for use by persons who wish to increase their satiety feeling, and as a result reduce their food intake." Your notification states that "...people suffering from nut allergies should be informed about the origin of PinnoThin™ FFA by proper labeling of the product, clearly revealing the origin of the product."

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate

commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b (a) (2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered to be adulterated under 21 U.S.C. 342(f) (1) (B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission and the agency has concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing "PinnoThin™ FFA" will reasonably be expected to be safe.

Your notification fails to adequately identify your new dietary ingredient, "PinnoThin™ FFA". Your notification provides a general description of your ingredient and a generic description of the process by which it is produced. However, the manufacturing process is not clearly described. In addition, the composition of your ingredient is described in a semi-quantitative way. Because the manufacturing process and the composition of your ingredient are not clearly described, the identity of your new dietary ingredient is unclear.

Your notification provides some information about the consumption of nuts from *Pinus koraiensis* and from other species of pine trees as well as information about the use in food of oils derived from pine nuts. However, your notification did not provide evidence of history of use of fatty acids derived from *Pinus koraiensis*.

Your notification provides the results of studies of pine nut oils (some of which were derived from *Pinus koraiensis*), pine seed oils and various other oils in rats, an *in vitro* mutagenicity test and information concerning the allergenicity of pine nuts or substances derived from pine nuts. However, it is unclear how your ingredient is qualitatively or quantitatively similar to the substances described in the information that you present as evidence of safety for your new dietary ingredient, or how that information is relevant to evaluating the safe use of your new dietary ingredient under the recommended conditions of use.

Your notification contains the results of a clinical study in which 3.0 g of PinnoThin™ was administered daily to 18 subjects for 2 weeks. However the submitted study results do not include a safety evaluation of the test material. In addition, the recommended conditions of use as stated in your notification are unclear. For example, your notification states that the recommended conditions of use will be to consume the ingredient to "provide a daily intake of 3.0 g PinnoThin™ FFA 30-60 min before the most substantial daily meal or 3.0 g of PinnoThin™ FFA between meals." is not stated. The maximum daily serving level and the number of recommended servings per day is not clearly stated. Furthermore, your notification does not contain a description of a dietary supplement product that would contain your new dietary ingredient. Therefore, it is unclear how the substance administered in the clinical study is qualitatively or quantitatively similar to a dietary supplement product containing your

new dietary ingredient or how the results of the submitted clinical study are relevant to evaluating the safe use of a dietary supplement product containing your new dietary ingredient under the recommended conditions of use.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that "PinnoThin™ FFA" when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of June 22, 2005. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter, please contact Linda S. Pellicore, Ph.D. at (301) 436-2375.

Sincerely yours,

Linda S. Pellicore

for Susan J. Walker, M.D.
Director
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety and Applied Nutrition

2005-3761

LIPID NUTRITION

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration

5100 Paint Branch Parkway
College Park, MD, 20740-3835
USA

Our ref.: LDe Tel.: +31 (0)75 629 2275 Wormerveer, June 17, 2005

NDI notification PinnoThin™ FFA

Dear Madam/Sir,

Pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act ("Act"), and FDA's implementing regulations at 21 C.F.R. § 190.6, Loders Croklaan B.V. ("Loders Croklaan") submits this New Dietary Ingredient Notification for PinnoThin™ FFA.

Please find included the notification dossier in 3-fold in which the safety for use as a dietary supplement has been substantiated for PinnoThin™ FFA.

Confidentiality:
Included is a summary of a non-published recent study I would like to have considered as confidential as long is it has not been published. I hereby ask to treat page 15 and 16 as confidential.

Sincerely yours,

Louis H.T Dederen
Safety & Regulatory Affairs Manager
Loders Croklaan, Lipid Nutrition
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

LIPID NUTRITION

LIPID NUTRITION

NEW DIETARY INGREDIENT NOTIFICATION FOR PinnoThin™ FFA

Loders Croklaan B.V.
Lipid Nutrition
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

Contact: Louis H.T. Dederen MSc.
Safety & Regulatory Affairs Manager
Telephone: +31-75 629 2275
Fax: +31-75 629 2217

June 17th, 2005

LIPID NUTRITION

NEW DIETARY INGREDIENT NOTIFICATION FOR PINNOTHIN™ FFA

Table of Contents

1. Justification

Pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act ("Act"), and FDA's implementing regulations at 21 C.F.R. § 190.6, Loders Croklaan B.V. ("Loders Croklaan") submits this New Dietary Ingredient Notification for PinnoThin™ FFA.

Based on the information described herein, including the history of use and other evidence of safety of pinenut oil, and pinenut oil fatty acids, and citations to published articles, Loders Croklaan concludes that dietary supplements containing PinnoThin™ FFA, when used under the conditions recommended or suggested by Loders Croklaan, will reasonably be expected to be safe.

2. Name and Address of the Manufacturer

Manufacturer:

Loders Croklaan B.V.
Hogeweg 1
1521 AZ Wormerveer
The Netherlands

Principal contact and correspondent:
L.H.T. Dederen, M.Sc.
Safety & Regulatory Affairs Manager
Telephone: +31-75 629 2275
Fax: +31-75 629 2217

Alternate contact:
Daniel R. Dwyer
Kleinfeld, Kaplan and Becker, LLP
1140 Nineteenth Street, NW
Washington, DC 20036
Telephone: 202-223-5120
Fax: 202-223-5619

Distributor:
Loders Croklaan,
Lipid Nutrition
24708 W. Durkee Road
Channahon, IL 60410-5249
USA.

Loders Croklaan is a bulk manufacturer of PinnoThin™; it does not manufacture finished dietary supplements containing PinnoThin™.

3. Name of the New Dietary Ingredient

The name of the new dietary ingredient is PinnoThin™ FFA.

4. Applications

PinnoThin™ FFA is suitable as ingredient in dietary supplements in various forms, including softgels, capsules, and supplement bars and similar products. Based on a human trial Loders Croklaan recommends that dietary supplement manufacturers formulate products to provide a daily intake of 3.0 g PinnoThin™ FFA 30 - 60 min before the most substantial daily meal or 3.0 g of PinnoThin™ FFA between meals (i.e. not after the last meal). The ingredient is intended for use by persons who wish to increase their satiety feeling, and as a result reduce their food intake.

5. Description of the New Dietary Ingredient

Chemically seen PinnoThin™ FFA can be characterized as Pine nut oil fatty acids. It actually is the natural mixture of fatty acids resulting from the hydrolysis (splitting) of Pine nut oil. The pine nuts applied are the peeled seeds of the Korean pine (*Pinus koraiensis*).

5.1. Chemistry

Typical fatty acid composition of PinnoThin™ FFA:

Fatty acid	Percentage		Percentage
C16:0	4.5	Total SAFA	7
C16:1	0.2	Total MUFA	26
C18:0	2	Total PUFA	67
C18:1	24	Total Trans	0.2
C18:2	46		
C18:2 5,9	2		
C18:3	0.2		
C18:3 5,9,12	16		
C20:0	0.3		
C20:1	1.3		
C20:2	0.9		
C20:3	1.6		
C22:0	0.1		
Other FA	0.4		

5.2. Specifications

PRODUCT DESCRIPTION:
PinnoThin™ FFA is a free fatty acid mixture from the vegetable pine seed oil of *Pinus koraiensis*.

TECHNICAL CHARACTERISTICS:

	Typical Values	
Free Fatty Acids	> 98.0 %	
Peroxide Value	max. 2.0	meq O_2/Kg

Appearance – light yellow, clear liquid at ambient, free from foreign odors and off flavors.

FATTY ACID COMPOSITION:

Total unsaturated fatty acids	≥	80 %
Mono unsaturated fatty acids		
Oleic acid	≥	20 %
Poly unsaturated fatty acids		
Linoleic acid	≥	45 %
Pinolenic acid	>	14 %
Saturated fatty acids	≤	10 %
Trans fatty acids	≤	2 %

ADDITIVES:

Mixed Natural Tocopherols	≥	250 ppm

PACKAGING:
PinnoThin™ FFA is packed under nitrogen and can be supplied in steel drums of 200 kg net.

KEEPABILITY / STORAGE:

PinnoThin™ FFA is stable for at least 18 months, if stored dry, in the unopened original packaging, preferably at a temperature of 10 – 20°C / 50 – 68°F, away from strong odors and not in direct sunlight.

5.3. Contaminant specifications

It is Loders Croklaan policy to ensure that all steps in the procurement and manufacturing process of products are carried out in a way which ensures that products are safe for use in foods and conform to accepted microbiological standards and relevant food legislation.

Monitoring

Good Manufacturing Practice (GMP), which includes HACCP risk analysis as well as a hygiene and transport standard, is applied throughout the manufacturing process. As a consequence, control takes place on a monitoring basis. Sampling schemes and analysis schedules are applied to raw materials and end-products as well as products in all stages of processing.

Microbiology

Total viable count	max.	1000/g	ISO 4833
Yeast	max.	10/g	ISO 7954
Moulds	max.	10/g	ISO 7954
Enterobacteriaceae	max.	10/g	ISO 7402
Salmonellae	absent in	25 g	ISO 6579
E-coli	absent in	1 g	ISO 7251

Trace metals and contaminants

Trace Metals			
Iron (Fe)	max.	0.5	ppm
Copper (Cu)	max.	0.05	ppm
Nickel (Ni)	max.	0.1	ppm
Cadmium (Cd)	max.	0.02	ppm
Mercury (Hg)	max.	0.05	ppm
Arsenic (As)	max.	0.1	ppm
Lead (Pb)	max.	0.1	ppm
Radio Activity	max.	600	Bq/kg
Mycotoxins			
Aflatoxine B1	max.	2	ppb
Aflatoxine B1+B2+G1+G2	max.	4	ppb

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PCB (IUPAC No.)			
28	<	10	ppb
52	<	10	ppb
101	<	10	ppb
118	<	10	ppb
138	<	10	ppb
153	<	10	ppb
163	<	10	ppb
180	<	10	ppb
Dioxine			
Vegetable oil	max.	0.75	pg WHO/ TEQ[1]/g fat
Fish oil	max.	2	pg WHO/ TEQ[1]/g fat
PAH			ppb
Total PAH	max.	25	ppb
Heavy PAH	max.	5	ppb
Benzo(a)pyrene	max.	1	
Pesticides/ Insecticides			
Nitrogen Pesticides			ppm
Dichlorbenil	max.	0.05	ppm
Diclofop - methyl	max.	0.05	ppm
Captafol	max.	0.05	ppm
Captan	max.	0.05	ppm
Procymidone	max.	0.05	ppm
Vinclozolin	max.	0.05	ppm
Propoxur	max.	0.05	ppm
Amitraz	max.	0.05	
Chlorine pesticides			ppm
HCB	max.	0.001	ppm
Alpha HCH	max.	0.001	ppm
Lindane	max.	0.001	ppm
Beta HCH	max.	0.001	ppm
Delta HCH	max.	0.001	ppm
Heptachlor	max.	0.005	ppm
Aldrin	max.	0.005	ppm
Chlordane	max.	0.005	ppm
pp DDE	max.	0.005	ppm
op DDE	max.	0.005	ppm
Endrin	max.	0.01	ppm
Methoxychlor	max.	0.01	ppm
Toxaphene	max.	0.01	ppm
Endosulfan	max.	0.005	ppm
PCB	max.	0.01	ppm
Phosphor Pesticides			
Azinphos - methyl	max.	0.01	ppm
Bromophos - ethyl	max.	0.01	ppm
Chlorfenvinphos	max.	0.01	ppm
Chlorpyriphos	max.	0.01	ppm
Diazinon	max.	0.01	ppm
Dichlorvos	max.	0.01	ppm
Disulfoton	max.	0.01	ppm
Ethion	max.	0.01	ppm
Fenitrothion	max.	0.01	ppm
Fensulfothion	max.	0.01	ppm
Fenthion	max.	0.005	ppm
Malathion	max.	0.01	ppm
Methidathion	max.	0.01	ppm
Mevinphos	max.	0.01	ppm
Naled	max.	0.01	ppm
Parathion - ethyl	max.	0.01	ppm
Parathion - methyl	max.	0.005	ppm
Phosphamidon	max.	0.01	ppm
Pirimiphos - ethyl	max.	0.01	ppm
Pirimiphos - methyl	max.	0.01	ppm
Sulfotep	max.	0.002	ppm
Trichlorphon	max.	0.01	ppm
Chlorpyrifos	max.	0.01	ppm
Chlorpyrifos-methyl	max.	0.01	ppm
Monocrotophos	max.	0.01	ppm
Omethoate	max.	0.01	ppm
Dimethoate	max.	0.01	ppm
Acephate	max.	0.01	ppm
Methamidophos	max.	0.01	ppm
Pyrethroides			
Fenvalerate	max.	0.05	ppm
Deltamethrin	max.	0.05	ppm
Cypermethrin	max.	0.05	ppm
Permethrin	max.	0.05	ppm

pp DDD	max.	0.005	ppm
op DDD	max.	0.005	ppm
pp DDT	max.	0.005	ppm
op DDT	max.	0.005	ppm
Heptachlor epoxyde	max.	0.005	ppm
Dieldrin	max.	0.01	

[1] WHO-PCDD/F-TEQ/ g fat

5.4. Process description

5.4.1. General

PinnoThin™ FFA is a natural mixture of the fatty acids derived by hydrolysis of refined Pinenut (*Pinus koraiensis*) oil.

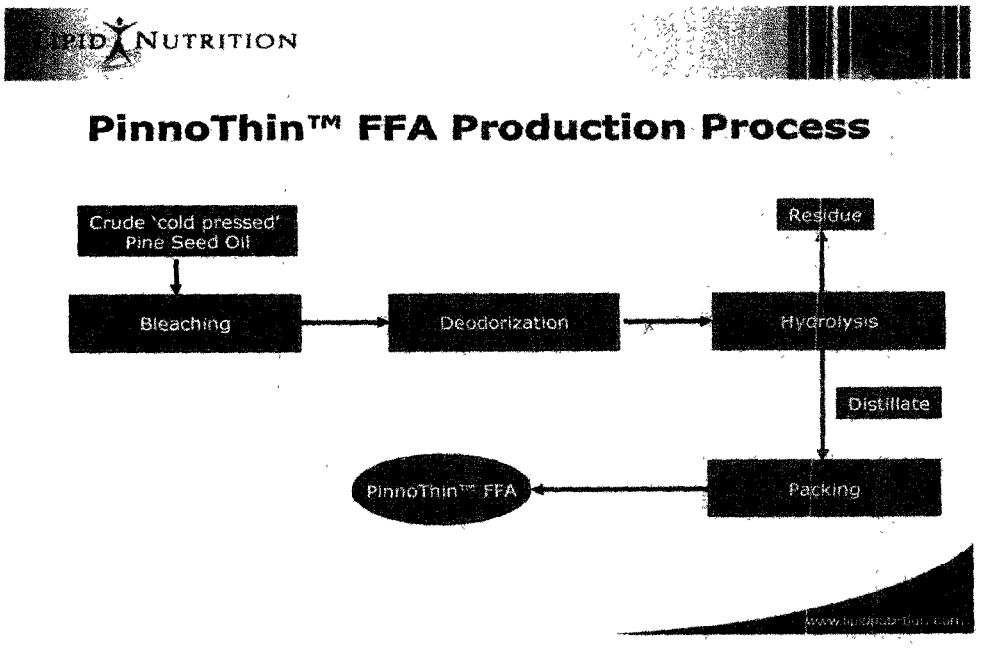
5.4.2. Process principle

Pinenut oil is produced by cold pressing of pine nuts of *Pinus koraiensis*. After pressing the pine nuts, the oil is bleached and deodorized, after which the triglycerides are split into fatty acids and glycerol.

To produce PinnoThin™ free fatty acids, the PinnoThin™ glycerides are fully hydrolyzed using specific GRAS food enzymes.

This process is consistent with generally applied food industry practices.

5.4.3. Process outline



5.4.4. Control of manufacture

Good Manufacturing Practice (GMP), which includes HACCP risk analysis as well as a hygiene and transport standard, is applied throughout the manufacturing process. As a consequence, control takes place within continuous monitoring. Sampling schemes and analysis schedules are applied to raw materials and end-products as well as products in all stages of processing.

Highly modern production facilities and electronic monitoring systems allow precise process control at all stages. Contact with air is excluded as the whole production of PinnoThin™ FFA as well as tapping in drums is carried out under nitrogen.

For the production of PinnoThin™ FFA a computer assisted sampling routine is used. All samples are logged in the system under a specific sample code for each process step. The necessary analyses, including the specifications the sample has to comply with, are defined within this code. It becomes immediately obvious whether the results are inside specifications or not. So, if need be, an instant intervention in the process is possible.

5.4.5. Control of the end-product PinnoThin™ FFA

The finished product is routinely tested to assure it meets specifications. These are set to ensure a high degree of purity consistent with its use as dietary supplement.

6. Occurence in the Diet

6.1. Pine nut oil fatty acids in the diet

Pine nuts have a long and safe history of consumption. They are well established in the food industry (FAO, 1998).[1] Except for being used in home cooking, Pine nuts are applied in well known foods and dishes like e.g. Pesto, roasted pine nut salads etc.. Pine nut oil is also available to the consumer, but is less commonly used due to the relatively high price of it, when compared to other vegetable oils applied for bakery, frying, cooking, and dressing of foods.

Pine nuts, especially those of *Pinus koraiensis*, are commonly eaten as savoury snack (nuts) during social events in China.

Pine nut oil consists of triglycerides of C14-C20 straight chain, saturated and unsaturated fatty acids. After consumption pine nut oil triglycerides are split in the stomach into glycerol and pine nut fatty acids, and also mono-, and diglycerides. These glycerides are also split into fatty acids and glycerol furtheron in the intestine.

As such, it can be stated that the metabolism of pine nut fatty acids is about similar to that of pine nut oil.

6.2. Level of Consumption

Pine nuts are a well consumed food ingredient all over the world. In the US 400-500 tons a year may be produced, while as much as 90% of the nuts used are imported from China (i.e. approx. 4500 tons a year) (see: http://www.pinenut.com/noha.htm).

Although the average level of consumption seems to be low when considering 300 million US citizens using 4500 tons a year, the actual intake by people using e.g. Pesto, a common Italian ingredient, will be much higher:

When considering the recipe of a standard Pesto:

125 g pine nuts
40 g fresh basilicum
30 g parmesan cheese
35 ml olive oil extra vergine
1 tea spoon salt
1 piece of garlic
it can be stated that pesto contains about 50% pinenut. Considering pine nuts to contain about 50% oil, a consumer using as little as 2 teaspoons of Pesto will already consume as much as 3 gram of pine nut oil.

In conclusion the average daily dietary intake of pine nut oil in the US can be estimated as low as 100 mg/day, but based on the assumption that the use is clearly not common to all US citizens, depending on cultural habits actual individual intakes will be much higher.

For instance Italians in Italy will generally eat pesto 1-2 times a week. The intake of pine nut oil resulting from this will be about 7,5 g in a serving. US citizens with Italian background may be expected to have comparable eating habits.

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[1] see: http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/X0453E/X0453e00.HTM

7. Safety determination of PinnoThin™ FFA

7.1. Literature data

Little has been published regarding the safety of pine nuts, pine nut oil, and pine nut fatty acids.

In a study to determine the influence of pine nut oil on immunization with intravenous ovalbumin Matsuo et al. (1996) fed rats a diet containing 10% (w/w) pine (*Pinus orientalis*) seed oil, evening primrose oil, or safflower oil in amounts equivalent to 1% of their body weight for 35 days (which corresponds with an average intake of 0.81 g pine nut oil a day for rats of 81 gram on the average). No changes were recorded in relative liver weight, weight gain, and growth, and adverse effects were neither recognized. When compared with Safflower oil intake the immunoglobulin production from splenic lymphocytes (both IgE and IgG) was increased by pine seed oil intake, while in the case of evening primrose oil intake the only the IgE production was found to be increased.

In a study of Asset et al. (1999) the antiatherogenic properties of *Pinus pinaster* (maritime pine) seed oil were studied. To this end, the effects of *P. pinaster* oil supplementation on lipoprotein levels and atherosclerotic lesions were compared to those of lard or sunflower oil in apolipoprotein E-deficient mice. Plasma total cholesterol ($P < 0.0001$) and VLDL + intermediary density lipoprotein (IDL)-cholesterol ($P < 0.0001$) levels were lower in mice fed *P. pinaster* and sunflower oil than in those fed the lard diet. In contrast, triglycerides ($P < 0.0001$) and VLDL + IDL-triglycerides ($P < 0.0001$) levels were higher in mice fed *P. pinaster* oil than sunflower oil or lard. The VLDL + IDL lipid composition of apolipoprotein E-deficient mice fed *P. pinaster* oil was intermediate between that of lard-fed transgenic mice and that of wild-type mice fed nonpurified diet. Using the Triton WR1339 method, the fractional catabolic rate of plasma triglycerides was found to be lower in mice fed *P. pinaster* oil ($P < 0.0001$) than sunflower oil or lard diet, suggesting a defective clearance of triglycerides in the *P. pinaster* group. Finally, the susceptibility of triglyceride-rich lipoproteins to in vitro lipoprotein lipase-mediated lipolysis was lower in the *P. pinaster* oil-fed group than in the lard-fed group. Despite the differences in VLDL + IDL level and lipid composition, the surface areas of aortic atherosclerotic lesions were not significantly different among mice fed *P. pinaster*, sunflower or lard diets. In conclusion, the results of the present study indicated that feeding *P. pinaster* oil had no better preventive effect on aortic atherosclerotic lesion extension in apolipoprotein E-deficient mice than other saturated or polyunsaturated fats.

Another study of Asset et al. was meant to assess the effect of vegetal oils obtained from *Pinus pinaster* and *P. koraiensis* seeds on plasma lipoprotein levels and apolipoprotein (apo) gene expression in rats. These oils contain two particular fatty acids of the delta5-unsaturated polymethylene-interrupted fatty acid (delta5-UPIFA) family: all-cis-5,9,12-1 8:3 (pinolenic) and/or all-cis-5,11,14-20:3 (sciadonic) acids. Rats were fed for 28 d a diet containing 5% (w/w) oil supplement. Two control diets were prepared to match the fatty acid composition of *P. pinaster* or *P. koraiensis* oils with the exception of delta5-UPIFA, which were replaced by oleic acid. *Pinus pinaster* seed oil decreased serum triglycerides by 30% ($P < 0.02$), very low density lipoprotein (VLDL)-triglycerides by 40% ($P < 0.01$), and VLDL-cholesterol by 33% ($P < 0.03$). *Pinus koraiensis* seed oil decreased serum triglycerides by 16% [not statistically significant (ns)] and VLDL-triglycerides by 21% (ns). Gel permeation chromatography and nondenaturating polyacrylamide gel electrophoresis showed a tendency of high density lipoprotein to shift toward larger particles in pine seed oil-supplemented rats. Finally, *P. pinaster* seed oil treatment was associated with a small decrease of liver

apoC-III ($P < 0.02$) but not in apoE, apoA-I, or apoA-II mRNA levels. The levels of circulating apo were not affected by pine seed oil supplementation. In conclusion, *P. pinaster* seed oil has a triglyceride-lowering effect in rats, an effect that is due to a reduction in circulating VLDL.

Sugano et al. (1992) fed rats a cholesterol-enriched diet containing 10% pine seed oil, linoleic acid (from safflower oil), or α-linolenic (from linseed oil) for 30 days. The dietary fats did not induce any changes in liver lipids, nor was any change observed in food intake, body weight gain or liver weight.
In the liver phoshatidylcholine, a lower linoleic acid level was found in the pine seed oil fed group, when compared to the group fed α-linolenic rich diet. On the other hand the proportion of arachidonic acid in the livers of the pine seed oil fed group, was higher than the level in the pinoleic acid group. Since the linoleic acid level in the diets is similar for the pine nut oil and the α-linoleic groups this finding suggests that pine nut oil, unlike α-linolenic acid, does not interfere with the desaturation process of linoleic acid.
In addition it was found that in this study the incorporation of linoleic acid in adipose triglycerides, is higher that that of α-linolenic acid, while pinolenic acid is incorporated at the lowest level.
There was no effect of treatment on aortic production of prostacyclin and no change occurred in ADP-stimulated platelet aggregation.
In another study presented in the same publication (Sugano et al., 1992) male spontaneously hypertensive rats (SHR) of 9 weeks old, were fed cholesterol free diets containing pine seed oil, evening primrose oil or safflower oil (10% of diet) for 8 weeks.
The age-related increase in systolic blood pressure in the spontaneously hypertensive rat was reduced in rat fed a 10% pine seed oil diet.

In another study of Sugano and his group (Sugano et al., 1994) the effects of dietary Korean pine (Pinus koraiensis)-seed oil containing a peculiar trienoic acid (cis-5,cis-9,cis-12-18:3, pinolenic acid, approximately 18%) on various lipid variables were compared in rats with those of flaxseed (Linum usitatissimum L.) oil, safflower (Carthamus tinctorius L.) oil and evening primrose (Oenothera biennis L.) oil under experimental conditions where the effects of different polyunsaturated fatty acids could be estimated. In Sprague-Dawley rats fed on diets containing 100 g fat and 5 g cholesterol/kg, the hypocholesterolaemic activity of pinolenic acid was intermediate between alpha-linolenic and linoleic acids. Analysis of the fatty acid composition of liver phosphatidylcholine indicated that, in contrast to alpha-linolenic acid, pinolenic acid does not interfere with the desaturation of linoleic acid to arachidonic acid. However, the effects on ADP-induced platelet aggregation and aortic prostacyclin production were comparable. When spontaneously hypertensive rats were fed on diets containing 100 g fat/kg but free of cholesterol, gamma-linolenic and pinolenic acids, as compared with linoleic acid, increased prostacyclin production and tended to reduce platelet aggregation. In addition, pinolenic acid attenuated the elevation of blood pressure after 5 weeks of feeding. Thus, the results of the present studies indicate the beneficial effects of pinolenic acid on various lipid variables.

7.2. Test on mutagenic potential of pine nut oil (Ames test)

Evaluation of the mutagenic activity of Pine nut oil (PSO) in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat), the Ames-test (NOTOX project 435779)

Pine nut oil (PSO) was tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains of *Salmonella typhimurium* (TA1535, TA1537, TA100 and TA98) and in the *Escherichia coli* reverse mutation assay with a tryptophan-requiring strain of *Escherichia coli* (WP_2uvrA). The test was performed in two

independent experiments in the presence and absence of S9-mix (phenobarbital and ß-naphthoflavone induced rat liver S9-mix).

Batch 5122 of PSO was tested as the free fatty acid form. It was a clear colourless liquid. The test substance was dissolved in ethanol.

In the dose range finding test, PSO was tested up to concentrations of 5000 µg/plate in the absence and presence of S9-mix in the strains TA100 and WP_2uvrA. PSO precipitated on the plates at dose levels of 1000 µg/plate and upwards. The bacterial background lawn was not reduced at any of the concentrations tested and no biologically relevant decrease in the number of revertants was observed.

Based on the results of the dose range finding test, PSO was tested in the first mutation assay at a concentration range of 10 to 1000 µg/plate in the absence and presence of 5% (v/v) S9-mix in tester strains TA1535, TA1537 and TA98. In the second mutation assay, PSO was tested at the same concentration range in the absence and presence of 10% (v/v) S9-mix in tester strains TA1535, TA1537, TA98, TA100 and WP_2uvrA. PSO precipitated on the plates at the top dose of 1000 µg/plate. The bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed.

PSO did not induce a dose-related, two-fold increase in the number of revertant (His^+) colonies in each of the four tester strains (TA1535, TA1537, TA98 and TA100) and in the number of revertant (Trp^+) colonies in tester strain WP_2uvrA both in the absence and presence of S9-metabolic activation. These results were confirmed in an independently repeated experiment.

In this study, the negative and strain-specific positive control values were within the NOTOX laboratory historical control data ranges indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

Conclusion:

Based on the results of this study it is concluded that Pine nut oil (PSO) is not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

7.3. Allergy data

The safety determination of PinnoThin™ FFA is merely based on the long safe history of use of pine nuts and pine nut oil. Except for few other studies on Pine nuts and pine nut oil, a number of allergy incidences have been reported in various pine nuts.

Ano et al.(2002) did report a case with a 10 year old boy diagnosed for seasonal rhinoconjuntivitis before, who reacted allergic to *Pinus pinea* nuts. Armentia et al.(1990) described a case of allergy to both pollen and nuts of *Pinus pinea.* De las Marinas (1998) reported about a case of pine nut allergy in a person with previously diagnosed Almond allergy. Then Moneret-Vautrin (1998) described a case of pine nut allergy in combination with a walnut allergy. Also cases with combined allergy to pine nut and hazelnut, walnut, cashew, and almond as well as those less frequently associated with allergies including pecan, chestnut, Brazil nut, pine nut, macadamia nut, pistachio, coconut, Nangai nut, and acorn have been described (Roux et al., 2003). Rubira et al.(1998) describing cases of allergy to pine nuts in children in the age from 12 months to 10 years, emphasizes that although pine nuts are very common food, until 1998 only 9 cases of allergy had been described in literature.

Although not specifically related to *Pinus koreaiensis* but to *Pinus pinea* the allergic agent has been recognized as protein 17-kDa (Garcia-Menaya et al., 2000; Ibanez et al., 2003). The allergen protein is sensitive to reduction (Rubira et al., 1998), and not

expected to occur in the oil phase in considerable quantities. Then during the splitting of the oil into fatty acids and glycerol, the allergen will preferably end up in the glycerol/water phase, and not in the PinnoThin™ FFA.

Based on the above studies it can be concluded that pine nut allergy is not expected to occur after consumption of PinnoThin™ FFA, since allergens are expected to be removed with the splitting process. Then it can be stated that the allergen residue that might occur in PinnoThin FFA will be that small, that no allergic reaction is to be expected. In addition one may wonder if the allergen will have allergic IgE binding capacities left after processing of PinnoThin™ FFA.

However, people suffering from nut allergies should be informed about the origin of PinnoThin™ FFA by proper labeling of the product, clearly revealing the origin of the product.

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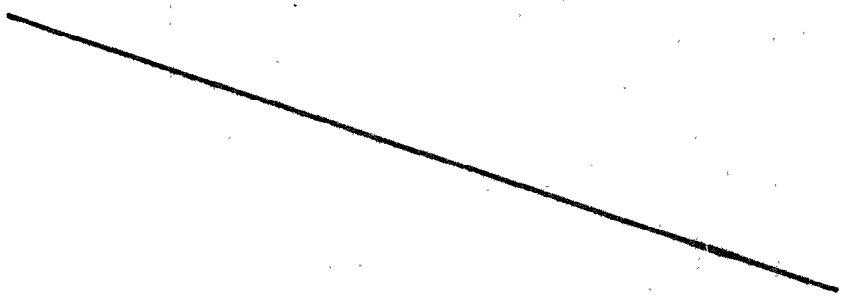
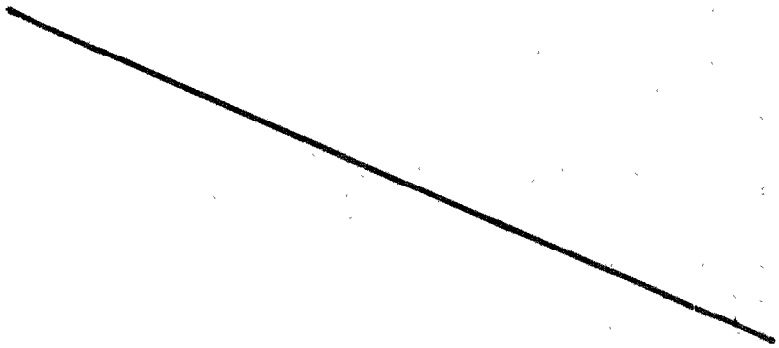
7.4. Human study

The effect of PinnoThin™ on markers of satiety (unpublished data)

Introduction



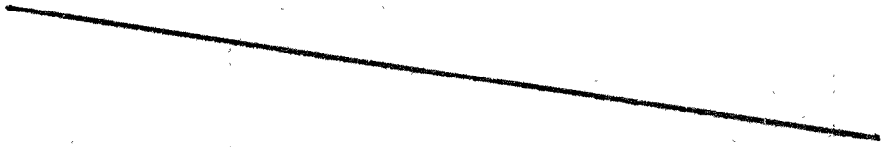
Study design



CONFIDENTIAL

Results.

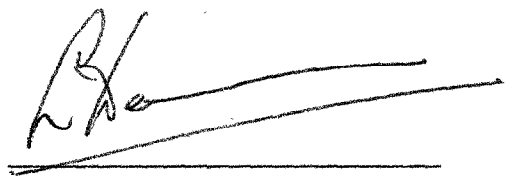
Conclusions



7.5. Conclusion

It can be stated that pine nut oil has a long history of safe consumption during which no safety items, other than incidental allergic reactions, have been reported so far. This has also been recognized by the FAO (1998)[2]. Based on that, and other facts included in this report it is concluded that PinnoThin™ FFA can be considered safe for use as a dietary supplement at the level proposed by Loders Croklaan.

V. SIGNATURE OF DESIGNATED PERSON



Louis H.T. Dederen
Safety & Regulatory Affairs Manager

[2] see: http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/X0453E/X0453e00.HTM

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